<u>REMARKS</u>

Claim Rejections Under 35 U.S.C. §101 and §112, First Paragraph

Claims 124-126 and 129-131 remain rejected under 35 U.S.C. §101 as allegedly lacking a specific, substantial and credible asserted utility or a well established utility. In her Answer dated October 12, 2005, the Examiner argues that "the data do not support the implicit conclusion of the specification that PRO341 genomic DNA shows a positive correlation with lung cancer, much less that the levels of PRO341 polypeptides would be diagnostic as such." The Examiner cites the following arguments and cites new references in support of these conclusions:

(1)   the genomic DNA encoding PRO341 had a ΔCt value for three out of fourteen lung tumor samples. Genomic DNA encoding PRO341 was not amplified in any of the fourteen colon tumor samples;

(2)   very few ΔCt values were obtained that were at least 2;

(3)   referring to three publications Livak *et al.*, Heid *et al.* and Pennica *et al.* cited in the Goddard Declaration, the Examiner says that "none of Livak *et al.*, Heid *et al.*, nor Pennica *et al.*, appear to indicate that an approximately 2-fold amplification of genomic DNA is significant in tumors;"

(4)   even if the data demonstrated a slight increase in copy number of PRO341 genomic DNA in primary tumors, such would not be indicative of a use of the claimed nucleic acids as diagnostic agents, since: (a) the data are not corrected for aneuploidy, (b) cancerous tissue is known to be aneuploid, (Sen (2000) cited); (c) Hittelman (2001) (**new reference cited**) teaches that precancerous lung epithelium is often aneuploid, therefore, based on the differential gene expression data one of ordinary skill would not conclude that PRO341 genomic DNA is amplified in cancerous lung epithelium more than damaged (non-cancerous) lung epithelium; and (d) an argument that PRO341 would still be a marker for at least precancerous or damaged lung epithelium would not prevail since it is "not suggested by the specification as originally filed and is not well-established in the prior art. (Emphasis added)."

(5)   the data for PRO341 genomic DNA have no bearing on the utility of the claimed PRO341 polypeptides. In order for PRO341 polypeptides to be overexpressed in lung tumors,

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amplified genomic DNA would have to correlate with amplified mRNA and the Examiner cited Pennica *et al.,* Hu *et al.* and Konopka *et al.* for support. The Examiner also cited **new references** LaBaer (2003), Gygi *et al.* (1999), Chen *et al.* (2002), Lian *et al.* (2001) and Fessler *et al.* (2002) for support to show that mRNA allegedly did not correlate well with amplified polypeptide levels.

The same arguments are cited in support of the rejection under 35 U.S.C. §112, first paragraph, for alleged lack of enablement for how to use the invention, since the claimed invention allegedly was not supported by either a credible, specific and substantial asserted utility or a well established utility.

Applicants disagree with each of the Examiner's arguments on a number for grounds.

<u>The Examiner's arguments are improper.</u>

The Examiner's arguments will be addressed in the order they are listed above.

(1)–(3) Regarding the Examiner's points recited in items (1), (2) and (3) above, Applicants have presented arguments why this rejection is improper in their Reply brief (now designated as a Response to non-final Office action) and maintain their position for the reasons cited therein. Applicants further request withdrawal of these rejections.

(4)   Regarding the Examiner's point on aneuploidy based on the cited references, Sen *et al.* and Hittelman *et al.,* Applicants have presented arguments in their Reply brief (now designated as a Response to non-final Office action) why both these references, in fact, support the Applicants' position that the PRO341 protein has at least one utility as a marker for cancerous or pre-cancerous tissue or damaged tissue. An additional review article by Hirsch *et al.* (<u>Clin. Cancer Research</u> 7: 5-22 (2001); copy enclosed) is also presented herein that supports the Applicants' assertion. Hirsch *et al.* present a review that focuses on the clinical perspectives of the biological knowledge of premalignant and early-malignant lesions and the potential advances for early diagnosis of lung cancer. Hirsch *et al.* summarize that "better understanding of the pathogenesis of lung cancer aroused renewed interest in morphological abnormalities that fall

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short of invasive carcinoma but may indicate initiation of carcinogensis" (page 6, column 1, last paragraph). Hirsch *et al.* add that "(n)umerous recent studies have indicated that lung cancer is not the result of a sudden transforming event in the bronchial epithelium but a multistep process in which gradually accruing sequential genetic and cellular changes result in the formation of an invasive (i.e. malignant tumor). Hirsch *et al.* reference the work of several authors (pages 17-22), many of which were performed well before the effective filing date of **July 9, 1998** of the instant application (specifically, see references 29-35 on page 18). Therefore, one skilled in the art of oncology, at the effective date of filing of the instant application, would have known, based on the teachings of the instant specification and the well-established art in lung cancer, how to make and use the instant PRO341 gene for the diagnosis of certain lung cancers, without undue experimentation.

(5)   In support of the rejection that "the data for PRO341 genomic DNA have no bearing on the utility of the claimed PRO341 polypeptides," the Examiner cited Pennica *et al.*, Konopka *et al.*, Hu *et al.*

Applicants disagree for the reasons set forth in their Reply Brief (now designated as a Response to Non-final Office Action). The test is whether it is more likely than not that gene amplification results in overexpression of the mRNA of the gene. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does <u>not</u> result in overexpression. Accordingly, Applicants maintain that the Examiner has not met the burden.

On the other hand, Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. The articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Appellants' Response filed July 7, 2004) collectively teach that <u>in general, gene amplification increases mRNA expression</u>. Accordingly, this rejection is improper.

The Examiner also cited several new references by LaBaer *et al.*, Gygi *et al.*, Chen *et al.*, Lian *et al.* and Fessler *et al.* in the Examiner's Answer of October 12, 2005.

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Again, Applicants have presented arguments why this rejection is improper based on the above cited references in their Reply brief (now designated as a Response to non-final Office action), and maintain their position for the reasons cited therein. In support of their assertion that there is "good correlation between mRNA levels and protein abundance," Applicants previously presented a Declaration by Dr. Paul Polakis (made of record in Appellants' Response filed July 7, 2004; for immediate reference, see Exhibit A of attached Declaration). However, the Examiner rejected the Polakis Declaration on the basis that "the instant specification provides no information regarding mRNA levels of PRO341....only gene amplification data was presented.....the declaration does not provide data such that the Examiner can independently draw conclusions." (Page 9 of the Final Action dated September 16, 2004).

Without acquiescing to the propriety of this rejection, and merely to expedite prosecution in this case, Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "A as such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA. Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

Applicants also present additional references herein that support the Applicants' assertion that there is "good correlation between mRNA levels and protein abundance." For instance, Papotti et al. (Diagn Mol Pathol. 9(1):47-57 (2000); copy enclosed) studied the somatostatin type 2 receptor (sst2) in 26 different neuroendocrine lung tumors. They investigated mRNA levels by RT-PCR and protein levels by immunohistochemistry using 2 different antibodies. The authors report that "in the majority of samples a good correlation between sst2 mRNA (as detected by RT-PCR) and sst2 protein expression (as detected by immunohistochemistry) was observed" (Abstract). The authors also performed in situ hybridization (ISH) in selected samples which

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"paralled the results obtained with the other techniques" (Abstract).

Walmer et al. (Cancer Res. 55(5):1168-75 (1995); copy enclosed) looked at lactoferrin mRNA and protein expression in endometrial adenocarcinomas and report that two thirds (8 of 12) of the samples examined overexpress lactoferrin. Walmer et al. also found that "this tumor-associated increase in lactoferrin expression includes an elevation in the mRNA and protein of individual cells" and that "serial sections of malignant specimens show(ed) a good correlation between the localization of lactoferrin mRNA and protein in individual epithelial cells by in situ RNA hybridization and immunohistochemistry" (Abstract).

Janssens et al. (Tumour Biol. 25(4):161-71 (2004); copy enclosed) evaluated the involvement of frizzled receptors (Fzds) in oncogenesis. They investigated mRNA expression levels in 30 different human tumor samples and their corresponding (matched) normal tissue samples by real-time quantitative PCR. Janssens et al. observed markedly increased Fzd5 mRNA levels in 8 of 11 renal carcinoma samples and indicate that "Western blot analysis of crude membrane fractions revealed that Fzd5 protein expression in the matched tumor/normal kidney samples correlated with the observed mRNA level" (Abstract).

Hahnel et al. (Breast Cancer Res Treat. 24(1):71-4 (1992); copy enclosed) studied expression of the pS2 gene in breast tissues by measuring mRNA levels using Northern blotting and protein levels by radioimmunoassay. Hahnel et al. indicate that "there was a good correlation between the two measurements, indicating that expression of the pS2 gene in breast tissues may be assessed by either method" (Abstract).

Kammori et al. (Int J Oncol.. 27:1257-63 (2005); copy enclosed) studied the expression of human telomerase reverse transcriptase (hTERT) gene and protein (besides estrogen and progesterone receptors) in breast tumors using in situ hybridization (ISH) for mRNA and immunohistochemistry (IHC) for the protein. They looked at 64 adenocarcinomas, 2 phyllode tumors and their adjacent normal breast tissues and found that hTERT mRNA was detected in 56 tumors but in neither of the 2 phyllode tumors whereas hTERT protein expression was detected by IHC in 52 tumors but in neither of the 2 phyllode tumors. The authors concluded that "there was a strong correlation between detection of hTERT gene expression by ISH and of hTERT

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protein by ICH in tissue specimens from breast tumors" (Abstract).

Maruyama *et al.* (Am. J. Pathol. 155:815-822 (1999); copy enclosed) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels" (Abstract). In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Bea *et al.* (Cancer Res. 61:2409-2412 (2001); copy enclosed) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in lymphoma samples. The authors examined BMI-1 protein expression in 31 tumors for which levels of gene amplification and mRNA expression had been determined. Bea *et al.* found that "[a] good correlation between BMI-1 mRNA levels and protein expression was observed in all types of lymphomas" (Abstract). Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Thus, together, the teachings within these references cited above overwhelmingly support the view that, in general, it is more likely than not that increases in DNA gene amplification will result in increases in mRNA which in turn results in increased protein expression in human cancers.

Also, in response to Gygi *et al.* which the Examiner cited in the Examiner's Answer, Applicants present a paper by Futcher *et al.*, (Mol. Cell. Biol. 19:7357-7368 (1999); copy enclosed). Futcher *et al.* completed a study similar to that of Gygi *et al.* but came to completely different conclusions from that of Gygi. Futcher *et al.* analyzed the yeast proteome using 2D gel

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electrophoresis and gathered quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene, as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that **"several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance"** (page 7360, col. 2; emphasis added). Futcher *et al.* further note that, despite generating broadly similar data, their conclusions markedly differ from the Gygi study and explain plausible reasons for such differing conclusions. Futcher *et al.* explain that this is, in part, a difference in viewpoint. For instance, Futcher *et al.* say that "(w)e believe that both mRNA abundance and codon bias are useful predictors of protein abundance. However, Gygi *et al.* feel that mRNA abundance is a poor predictor of protin abundance and that "codon bias is not a predictor of either protein or mRNA levels.... Gygi *et al.* focus on the fact that the correlations of mRNA and codon bias with protein abundance are far from perfect while we focus on the fact that, considering the wide range of mRNA and protein abundance and the undoubted presence of other mechanisms affecting protein abundance, the correlations are quite good" (page 7367, col. 1). Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots. In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather

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than indicating a truly poor correlation in vivo" (page 7367, col. 2). Futcher *et al.* further point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* first note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) to measure the covariance of mRNA and protein abundance. Futcher *et al.* point out that "the r_p correlation is a parametric statistic and so requires variates following a bivariate normal distribution; that is, it would be valid **only if both mRNA and protein abundances were normally distributed**" (page 7367, col. 1; emphasis added). As the authors disclose, "both distributions are very far from normal," and thus "a calculation of r_p is inappropriate" (page 7367, col. 1).

Accordingly, the results of Futcher *et al.* demonstrate "a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2). Further, Futcher *et al.* show that when corrected for an inappropriate statistical analysis and systematic error in the measurement of low abundance proteins, the data of Gygi *et al.* also meets the "more likely than not standard" and shows that a positive correlation exists between mRNA levels and protein levels.

In summary, the teachings of the new Polakis II Declaration, the new references and previously submitted references, Declarations and arguments overwhelmingly support the view that, in general, it is more likely than not that increases in DNA gene amplification will result in increases in mRNA which in turn results in increased protein expression in human cancers. Applicants further respectfully submit that the Examiner has not established a *prima facie* showing of lack of utility based on the references cited in the Examiner's answer and therefore, the Patent Office has failed to meet its initial burden of proof. Accordingly, this rejection under 35 U.S.C. §101 and §112, first paragraph, should be withdrawn.
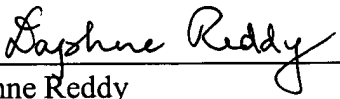
CONCLUSION

For the reasons given above, Applicants submit that the gene amplification assay disclosed in Example 170 of the specification, and the advanced state of the art in oncology, provide at least one patentable utility for the PRO341 polypeptides of Claims 124-126 and 129-

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131, and that one of ordinary skill in the art would understand how to use the claimed polypeptides and would have found such testing routine and <u>not</u> 'undue.' Therefore, Claims 124-126 and 129-131 meet the requirements of 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2730 P1C1**).

Respectfully submitted,

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